Nutritional Factors and Preservation of C-Peptide in Youth With Recently Diagnosed Type 1 Diabetes

SEARCH Nutrition Ancillary Study

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OBJECTIVE—To test the novel hypothesis that nutritional factors previously associated with type 1 diabetes etiology or with insulin secretion are prospectively associated with fasting C-peptide (FCP) concentration among youth recently diagnosed with type 1 diabetes.

RESEARCH DESIGN AND METHODS—Included were 1,316 youth with autoantibody-positive type 1 diabetes who participated in the SEARCH for Diabetes in Youth study (baseline disease duration, 9.9 months; SD, 6.3). Nutritional exposures included breastfeeding and age at introduction of complementary foods, baseline plasma long-chain omega-3 fatty acids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), vitamin D, vitamin E, and, from a baseline food frequency questionnaire, estimated intake of the branched-chain amino acid leucine and total carbohydrate. Multiple linear regression models were conducted to relate each nutritional factor to baseline FCP adjusted for demographics, disease-related factors, and other confounders. Prospective analyses included the subset of participants with preserved β-cell function at baseline (baseline FCP ≥0.23 ng/mL) with additional adjustment for baseline FCP and time (mean follow-up, 24.3 months; SD, 8.2; n = 656). FCP concentration was analyzed as log(FCP).

RESULTS—In adjusted prospective analyses, baseline EPA (P = 0.02), EPA plus DHA (P = 0.03), and leucine (P = 0.03) were each associated positively and significantly with FCP at follow-up. Vitamin D was unexpectedly inversely associated with FCP (P = 0.002).

CONCLUSIONS—Increased intake of branched-chain amino acids and long-chain omega-3 fatty acids may support preservation of β-cell function. This represents a new direction for research to improve prognosis for type 1 diabetes.

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After clinical diagnosis of type 1 diabetes, patients commonly retain limited capacity to secrete insulin for several months or years (1). Preservation of β-cell function, measured by C-peptide concentration (2), has been associated with lower risk for hypoglycemic events as well as lower HbA_{1c} and less frequent microvascular complications (3). Therefore, identification of interventions to protect against β-cell loss after onset of type 1 diabetes may improve prognosis. We hypothesized that nutritional factors previously identified as potentially protective against development of diabetes autoimmunity or type 1 diabetes (4), or those associated with mechanisms related to insulin production (5), also may be useful toward preservation of β-cell function. The rationale for our hypothesis is based on the notion that the etiology of type 1 diabetes is multifactorial and that any given protective nutritional factor may not be sufficient to protect against disease onset but still may influence disease progression after clinical diagnosis.

Infant feeding practices, including breastfeeding (6,7) and timing of introduction of complementary foods (8,9), have been associated with the development of diabetes autoimmunity or type 1 diabetes. Additional work suggests that vitamins D and E and long-chain polyunsaturated omega-3 fatty acids (10,11) may have protective effects against autoimmune-mediated diabetes. Branched-chain amino acids, particularly leucine, can promote insulin secretion (5), and total carbohydrate intake drives postprandial glucose excursion and thus contributes substantially to insulin demand (12). The aim of the present analysis was to prospectively evaluate each of these nutritional exposures in relation to fasting C-peptide (FCP) in a large cohort of youth with recently diagnosed type 1 diabetes, with ~2 years of follow-up. Thus, based on published literature, the following three sets of nutritional exposures were included: reported history of

infant feeding practices; baseline plasma biomarkers of selected nutrients; and baseline estimated intake of selected nutrients from a food frequency questionnaire. Results can inform the design of new studies to establish the efficacy of nutritional interventions to support β-cell function in youth with recently diagnosed type 1 diabetes.

RESEARCH DESIGN AND METHODS

Overview

Data were derived from the longitudinal cohort portion of the multicenter SEARCH for Diabetes in Youth study and additional data were collected by the SEARCH Nutrition Ancillary Study (SNAS), which was designed to test hypotheses related to potential nutritional determinants of sustained β-cell function among youth with type 1 diabetes. The parent SEARCH study (13) has ascertained cases of childhood diabetes among youth younger than age 20 years who were incident in 2002 or later; ascertainment and data collection are ongoing. SNAS was funded in 2008, and data collection was completed in 2011. Both studies were reviewed and approved annually by the local institutional review boards that had jurisdiction over the local study population and complied with the Health Insurance Portability and Accountability Act.

Data collection

All SEARCH participants who were incident in 2002–2005 and who completed a baseline study visit were invited for follow-up visits at ~12 and 24 months after the baseline visit. Written informed consent and assent were obtained according to the guidelines established by the local institutional review board at the beginning of each study visit.

Data on treatment regimen, including type of insulin, total daily insulin dose, frequency of insulin injections, or use of continuous subcutaneous insulin infusion (insulin pump), were obtained by interview with the parent for participants younger than age 18 years or from the participants who were 18 years of age or older. Fasting blood samples were obtained under conditions of metabolic stability, defined as no episode of diabetic ketoacidosis during the previous month. FCP was determined by a two-site immunoenzymetric assay (Tosoh AIA; Tosoh Bioscience, San Francisco, CA). The assay sensitivity was 0.05 ng/mL. Samples were analyzed for glutamic acid decarboxylase-65 (GAD65) and insulinoma-associated-2 (IA-2) diabetes autoantibodies using a standardized protocol and a common serum calibrator (14). The cut-off values for positivity were 33 NIDDK units (NIDDKU)/mL for GAD65 and 5 NIDDKU/mL for IA-2 (14). HbA_{1c} was measured in whole blood with an automated nonporous ion-exchange high-performance liquid chromatography system (model G-7; Tosoh Bioscience, Montgomeryville, PA). Human leukocyte antigen (HLA) class II genotyping was performed with a PCR-based sequence-specific oligonucleotide probe system (15). HLA genotypes were categorized as susceptible (DR3/4, DR4/4, DR4/8, DR4/1, DR 4/13, DR 3/3, DR 3/9, DR 4/9, and DR 9/9 genotypes) and not susceptible (15).

For youth 10 years of age or older, usual dietary intake was assessed by a food frequency questionnaire (FFQ) from a previous week that included foods to reflect the ethnic and regional diversity represented by the cohort, as previously described (16). In brief, the SEARCH FFQ is semiquantitative, with ~85 food lines queried for weekly frequency of consumption for each food item. Portion sizes for each food or beverage are assessed in a manner relevant to that particular item, e.g., number of eggs or small, medium, and large for food in bowls using a two-dimensional food portion visual. The FFQ was self-administered by the participant with staff instruction and support as needed. Parental input was allowed for information the participant was unable to recall or did not know (e.g., type of milk used). The nutrient and portion-size database for this instrument was established using the Nutrition Data Systems for Research database (version 4.05/33, 2002; Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN) and industry sources. Although the FFQ provided data on three branched-chain amino acids (leucine, isoleucine, and valine), we analyzed only leucine because the correlation among these three amino acids was so high ($r > 0.95$) that unique information across the three amino acids could not be discerned.

Data collection

For SEARCH participants with type 1 diabetes diagnosed in 2002–2005, additional data collected for SNAS included an infant diet history; using frozen samples obtained from the baseline SEARCH examination stored at −80°C, plasma measures of selected nutrients were obtained including vitamin D, vitamin E, and fatty acids.

Infant diet history was assessed through maternal self-report with a questionnaire modeled after that used by Norris et al. (17). The questionnaire was structured as a temporal grid, representing the child's age at specific intervals (first 3 days of life, first week, and each month through 12 months). In addition to querying breastfeeding and formula feeding, respondents were asked when listed food and beverage items were introduced on a regular basis defined as "at least once per week." Infant foods queried separately include breast milk, formula, cow milk, soy milk, fruit and fruit juice, cereals, vegetables, beef, fish, cod liver oil, and vitamins.

Nutrient biomarkers

The concentration of 25-hydroxyvitamin D (25-OHD) was measured in plasma using the direct competitive chemiluminescence immunoassay developed by Dia Sorin (Stillwater, MN) (UNC detectable range, 5–320 nmol/L; intra-assay coefficient of variation, 11.0%) based on a linkage between specific vitamin D antibody–coated magnetic particles and an isoluminol derivative. This method uses an antibody as a primary binding agent and measures both 25-OHD2 and 25-OHD3 (18). There were 13 with vitamin D levels below the detectable limit. For the purpose of analysis, these values were imputed as 4.9 nmol/L. Plasma concentration of α-tocopherol was assayed by high-performance liquid chromatography using the method of Cheng et al. (19) (interassay coefficient of variation <5%).

The utility of the fatty acid profile of plasma phospholipids to measure dietary fatty acid intake has been validated relative to the capacity of these nutrient biomarkers to predict the specific fatty acid intake of highly controlled test diets (20,21). For fatty acid measures, total lipids were extracted from plasma by the Bligh-Dyer method and phospholipids were separated from all other lipids by one-dimensional thin-layer chromatography. The phospholipid extract was then saponified and transmethylated using the method of Tacconi and Wurtman (22). Gas chromatography was performed on samples dissolved in undecane using conditions modified from Lemaitre et al. (23). Data were analyzed

with ChemStation Firmware A.01.09 (Agilent). The coefficients of variation (%) in the quality control pool samples of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) were 11.5% and 9.9%, respectively.

Subject inclusion

Included were youth with type 1 diabetes defined by physicians as type 1, type 1a, or type 1b diabetes, plus a positive test for at least one diabetes autoantibody (GAD65 or IA-2) and baseline measurement of FCP (n = 1,316). For longitudinal analyses, we restricted the analytic sample to those who met the Diabetes Control and Complications Trial–based definition of preserved β-cell function of FCP ≥0.23 ng/mL (1,3) at the baseline examination and for whom at least one follow-up value of FCP was available (n = 656). Thus, the longitudinal analyses specifically addressed the question of whether variability in nutritional exposures (early infant diet or usual diet as of the baseline visit) predicts variability in follow-up FCP among those for whom continued preservation of β-cell function was possible. For individuals with two follow-up FCP values available, we included only the most recent value. Sample sizes varied according to availability of nutritional exposure data (Table 1).

Statistical analyses

Initial descriptive analyses were conducted using ANOVA to examine how the exposures varied according to categories of baseline FCP (≤0.23 ng/mL; >0.23 to ≤0.65; and ≥0.65) to assess the possibility of threshold effects. Because threshold effects were not evident (data not shown), study hypotheses were evaluated using linear regression to predict FCP, which was log-transformed to provide better model fit. Initially, baseline unadjusted models were fit, followed by models adjusted for potential confounders both to minimize bias in the results and to improve precision of estimates of association. For consistency, the same potential confounders were included in models conducted separately for each nutritional exposure, with minor exceptions noted. Potential confounders were variables shown to be associated with preserved β-cell function (1) or variables that may be associated with the nutritional exposures, such as demographics (age, parental education, race/ethnicity, sex, clinic), diabetes-related variables (duration, insulin regimen, insulin dose per kilogram, fasting glucose, HbA_{1c}), and saturated fat (specifically, plasma saturated fatty acids, except for models of exposures based on the FFQ in which saturated fat [grams] from the FFQ was used). For baseline nutritional exposures derived from the FFQ (total carbohydrate and leucine), models were additionally adjusted for total calories. For vitamin D, models also were adjusted for season of the year and for BMI (z-score) because obese individuals have lower levels of 25(OH)D than lean individuals, which may be related to sequestering and storage of vitamin D in adipose tissue (24). To assess consistency between the baseline and longitudinal samples, baseline analyses were conducted for the full analytic sample and were repeated for the subset of participants included in the longitudinal analyses.

To address the primary study hypotheses, longitudinal analyses used linear regression models to predict follow-up log FCP. Primary results were derived from models that included the same set of potential confounders used in cross-sectional models with the additional adjustment for baseline FCP and time between the baseline and follow-up examinations. Finally, for both cross-sectional and longitudinal models, an interaction term was added to the adjusted models to test for potential effect modification by HLA risk group, although it was not retained because it was not significant in all models. Statistical significance was established when $P < 0.05$.

RESULTS—Baseline characteristics of participants included in the initial baseline sample (n = 1,316) and the subset of those included in the longitudinal analyses (n = 656) are shown in Table 1. By design, although the full sample included 28.5% of youth with type 1 diabetes who had already lost all meaningful β-cell function according to the Diabetes Control and Complications Trial criteria of FCP ≤0.23 ng/mL, all of those included in the longitudinal subset had preserved β-cell function at the time of the baseline examination. At follow-up, 58% of those in the longitudinal subset had lost β-cell function.

Table 2 contains the results of the initial baseline associations for the three sets of nutritional exposures in relation to baseline FCP for both the full sample and the longitudinal subset. Lower plasma vitamin E was associated with higher baseline FCP in the adjusted model in the longitudinal subset only. Higher plasma EPA was significantly associated with higher baseline FCP, but only in the unadjusted model for entire sample. Higher plasma DHA and the sum of EPA and DHA were associated significantly with higher baseline FCP in both the full sample and in the longitudinal subset, but this cross-sectional association was not retained after adjustment for potential confounders. From the FFQ, higher estimated intake of leucine was associated significantly with higher baseline FCP in the unadjusted model for the longitudinal sample.

The results of the longitudinal analyses of associations between each of the nutritional exposures and follow-up FCP are presented in Table 3. Surprisingly, higher levels of plasma vitamin D at baseline were associated significantly with lower levels of follow-up FCP, even after adjustment for covariates. Higher levels of plasma EPA, DHA, and their sum at baseline were each associated with significantly higher follow-up FCP in unadjusted models (all $P \leq 0.01$). After adjustment for potential confounders, EPA and the sum of EPA and DHA remained significantly positively associated with follow-up FCP (P = 0.02 and 0.03, respectively). Participants with higher estimated intake of leucine at baseline had higher follow-up FCP after adjustment for potential confounders (P = 0.03).

To put the statistically significant findings in the clinical context in which the average change in FCP in the longitudinal subset was a decrease of 57% (Table 1), we estimated the difference in follow-up FCP that would be predicted by a 1-SD difference in the nutritional exposure at baseline after adjustment for confounding. Unexpectedly, a 1-SD higher baseline plasma vitamin D level was associated with a 12.6% lower FCP at follow-up. Consistent with the hypothesized effects, a 1-SD higher plasma EPA level and EPA plus DHA level at baseline were associated with 9.2% and 8.9% higher follow-up FCP, respectively. A 1-SD higher estimated intake of leucine was associated with 5.7% higher follow-up FCP.

CONCLUSIONS—From a large, diverse cohort of youth with type 1 diabetes confirmed by the presence of diabetes autoimmunity, findings suggest that selected nutritional factors may act either to enhance or to reduce β-cell decline over a

Table 1—*Baseline characteristics of SEARCH 2002–2005 incident case analytic sample and the subset of those included in longitudinal analyses*

	All cases		Longitudinal analytic subset (baseline FCP >0.23 ng/mL)	
	N	Mean (SD) or %	*N*	Mean (SD) or %
Demographic and clinical characteristics				
Age, years	1,316	11.1 (4.0)	656	11.5 (3.7)
Race/ethnicity				
Non-Hispanic white	1,025	77.9	513	78.2
African American	121	9.2	59	9.0
Hispanic	143	10.9	73	11.1
Asian/Pacific Islander	15	1.1	6	0.9
American Indian	6	0.5	2	0.30
Other	6	0.5	3	0.5
Sex, % female	642	48.8	311	47.4
Parental education				
Less than high school	56	4.3	25	3.8
High school graduate	188	14.4	86	13.2
Some college/Associate's degree	440	33.6	226	34.6
Bachelor's degree or more	624	47.7	316	48.4
Diabetes duration at initial visit, months	1,316	9.9 (6.3)	656	7.9 (5.6)
Insulin regimen				
Pump	114	8.8	47	7.3
Long + short/rapid insulin, ≥3 times/day	409	31.5	235	36.4
Long + any other combination, ≥2 times/day	90	6.9	36	5.6
Any combination of insulin excluding long, ≥3 times/day	198	15.3	89	13.8
Any insulin used once/day or any insulin combination excluding long, twice/day	486	37.5	239	37.0
Insulin dose, units/kg	1,257	0.64 (0.32)	634	0.56 (0.3)
HbA_{1c}, %	1,310	7.72 (1.53)	654	7.4 (1.3)
Fasting glucose, mg/dL	1,308	171 (77)	653	156.9 (65)
BMI z-score	1,281	0.56 (0.97)	641	0.61 (0.95)
Fasting C-peptide				
Baseline FCP, ng/mL	1,316	0.69 (0.65)	656	0.91 (0.7)
Follow-up FCP, ng/L	909	0.31 (0.40)	656	0.37 (0.44)
Change in FCP from baseline until follow-up, ng/mL	909	−0.40 (0.59)	656	−0.53 (0.63)
Percent change in FCP from baseline until follow-up	909	−41.9 (90.9)	656	−57.4 (37.8)
Baseline FCP category, ng/mL				
≤0.23	375	28.5	0	0
>0.23– 0.65	440	33.4	307	46.8
>0.65	501	38.1	349	53.2
Follow-up fasting C-peptide category, ng/mL				
≤0.23	613	67.4	378	57.6
>0.23–0.65	181	19.9	168	25.6
>0.65	115	12.7	110	16.8
Infant feeding exposures				
Breastfeeding				
Never	259	28	136	26.9
<6 months	275	29.8	155	30.7
≥6 months	390	42.2	214	42.4
Age at introduction of complementary foods, months				
Any solid food	960	4.64 (2.11)	524	4.62 (2.17)
Any dairy (including formula)	870	4.89 (4.24)	468	4.71 (4.18)

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Table 1—*Continued*

	All cases		Longitudinal analytic subset (baseline FCP >0.23 ng/mL)	
	N	Mean (SD) or %	*N*	Mean (SD) or %
Any vegetable (excluding potatoes)	929	6.55 (2.16)	505	6.57 (2.18)
Gluten-containing cereal	935	6.81 (2.75)	515	6.85 (2.8)
Baseline nutritional exposures: biomarkers				
Vitamin D (plasma 25-OHD), nmol/L	1,145	58.3 (33.4)	591	60.0 (34.6)
Vitamin E (plasma α-tocopherol), μg/mL	1,005	6.2 (3.04)	520	5.94 (2.82)
EPA, plasma phospholipid percent weight	820	0.41 (0.25)	398	0.42 (0.27)
DHA, plasma phospholipid percent weight	827	2.45 (0.83)	400	2.45 (0.84)
EPA + DHA	820	2.86 (0.97)	398	2.87 (1.01)
Baseline nutritional exposures: estimated intake from FFQ				
Leucine, g/1,000 kcal, from FFQ*	730	3.21 (0.51)	397	3.24 (0.5)
Carbohydrate, g/1,000 kcal, from FFQ*	730	115.9 (18.3)	397	115.0 (18.2)

SEARCH 2002–2005 incident case analytic sample ($n = 1,316$) and the subset of those included in longitudinal analyses ($n = 656$) with baseline FCP ≥0.23 ng/mL and follow-up data available. *FFQ administered to youth 10 years of age or older.

period of ~2 years after clinical diagnosis of type 1 diabetes. Unexpectedly, baseline plasma concentration of vitamin D was associated inversely with FCP concentration at follow-up. However, consistent with study hypotheses, intake of long-chain omega-3 polyunsaturated fatty acids and branched-chain amino acids may prospectively support sustained β-cell function.

As previously reported from the SEARCH cohort, we have shown that among individuals with diabetes who are positive for diabetes autoimmunity, younger age at diagnosis, non-Hispanic white race/ethnicity, male sex, higher baseline HbA_{1c}, and lower baseline BMI z-score were independently associated with significantly lower baseline FCP level (25). The predicted rate of decline in FCP levels among these youth was 4.1% (95% CI, 3.9–4.3) per month, regardless of age at diagnosis, sex, race/ethnicity, and HLA risk status. When baseline diabetes autoantibodies positivity status was added to the model, the presence of two diabetes autoantibodies versus one diabetes autoantibody was the only variable significantly associated with faster decline in FCP levels ($P < 0.0001$): estimated 4.4% per month (95% CI, 4.1–4.7) in those with double autoantibody positivity versus 3.7% per month (95% CI, 3.4–4.0) in those with single autoantibody positivity. This suggests that factors that impact on the underlying autoimmune processes may, in turn, impact on rate of β-cell decline. The present finding demonstrates that selected nutritional factors may be important.

Across exposures, occasional differences were observed between baseline and prospective analyses. We focus attention on results from the prospective analyses for three reasons. First, this is consistent with the SNAS a priori hypotheses and study design. Second, inclusion of baseline FCP in the prospective analyses allowed for improved precision by accounting, at least in part, for other unmeasured factors that impact directly on both baseline and follow-up FCP in individuals, such as genetics or other biological determinants of FCP and change in FCP. Third, the correct temporal sequence is established in the prospective analyses, whereas this is not true of the baseline analyses.

Infant feeding exposures

Infant feeding may contribute to the development of type 1 diabetes, but the research evidence is equivocal (6,7) and may depend on genetic susceptibility (26). In the current study, we found no evidence that breastfeeding was associated with FCP at either baseline or follow-up. We also evaluated the timing of introduction of selected foods to the infant diet because these may alter risk for autoimmunity and type 1 diabetes (8,9,27), although again the literature is mixed and our findings were negative.

Baseline nutritional exposures: biomarkers

Cod liver oil in infancy has been inversely associated with type 1 diabetes (10), which could reflect a protective effect of one or more of the major constituents of cod liver oil, i.e., vitamin D and the omega-3 fatty acids derived from fish, including EPA and DHA. Vitamin D or genetic variations of the vitamin D receptor may protect against inflammation and autoimmunity (28); however, studies focused on type 1 diabetes have yielded mixed results (29,30). Unexpectedly, in our longitudinal analyses, baseline plasma vitamin D was significantly inversely associated with follow-up FCP. Results were verified by review of unadjusted scatter plots, confirmation of model fit, and evaluation of potential effect modifiers (BMI, duration of diabetes, baseline FCP, and HLA), and the finding of an inverse association was confirmed. Our study was limited in that measurement of the active form of vitamin D [$1,25(OH)_2D_3$] is not feasible in epidemiologic studies, and we did not have measures of variation in the vitamin D receptor and other vitamin D metabolism genes. Of note, two small clinical trials failed to demonstrate benefit of vitamin D supplementation on sustained β-cell function (31,32); thus, taken together with our findings, it is unlikely that vitamin D improves β-cell preservation.

The role of EPA and DHA on immune function appears to differ substantially according to disease state, such that studies of disease-specific outcomes are required (33). Animal models show protection by these fatty acids against development of type 1 diabetes (34); however, a recent study of nutritional status in a large cohort of youth failed to identify a protective effect on β-cell

Table 2—*Cross-sectional associations of nutritional exposures with log fasting C-peptide for SEARCH 2002–2005 incident cases and the subset of cases included in longitudinal analyses, unadjusted and adjusted* for potential confounders*

		All cases N = 1,316†		Longitudinal analytic subset (baseline FCP >0.23 ng/mL) N = 656†	
	Model	Beta coefficient (SE)	P	Beta coefficient (SE)	P
Infant feeding exposures					
Breastfeeding	Unadjusted		0.26 (overall)‡		0.70 (overall)‡
	Never	0.10 (0.07)		0.06 (0.07)	
	<6 months	0.09 (0.07)		0.02 (0.06)	
	≥6 months	0 (referent)		0 (referent)	
	Adjusted		0.051 (overall)‡		0.43 (overall)‡
	Never	0.15 (0.07)		0.06 (0.08)	
	<6 months	−0.03 (0.07)		−0.05 (0.08)	
	≥6 months	0 (referent)		0 (referent)	
Age at introduction of complementary food, months					
Any solid food	Unadjusted	−0.014 (0.013)	0.29	0 (0.012)	0.97
	Adjusted	0.006 (0.013)	0.67	0.026 (0.014)	0.07
Any dairy (including formula)	Unadjusted	−0.0073 (0.0069)	0.29	0.0001 (0.0068)	0.99
	Adjusted	−0.0031 (0.007)	0.65	0.011 (0.0079)	0.16
Any vegetable (excluding potatoes)	Unadjusted	−0.0063 (0.0131)	0.63	0.0035 (0.0125)	0.78
	Adjusted	0.0073 (0.013)	0.57	0.0181 (0.0138)	0.19
Gluten-containing cereal	Unadjusted	0.0083 (0.0102)	0.41	0.0052 (0.0096)	0.59
	Adjusted	0.0167 (0.0101)	0.10	0.0192 (0.0108)	0.08
Baseline nutritional exposures: biomarkers					
Vitamin D (plasma 25-OHD), nmol/L	Unadjusted	−0.0013 (0.0008)	0.10	−0.0015 (0.0007)	0.05
	Adjusted	0.0014 (0.0008)	0.07	0.0008 (0.0008)	0.30
Vitamin E (plasma α-tocopherol), μg/mL	Unadjusted	−0.015 (0.009)	0.11	−0.013 (0.01)	0.18
	Adjusted	−0.014 (0.008)	0.09	−0.024 (0.01)	0.01
EPA, plasma phospholipid percent weight	Unadjusted	0.231 (0.117)	0.049	0.067 (0.11)	0.54
	Adjusted	0.03 (0.096)	0.75	−0.019 (0.098)	0.84
DHA, plasma phospholipid percent weight	Unadjusted	0.088 (0.0346)	0.01	0.0897 (0.0354)	0.01
	Adjusted	0.017 (0.03)	0.57	−0.002 (0.033)	0.96
EPA + DHA	Unadjusted	0.078 (0.030)	0.008	0.068 (0.030)	0.02
	Adjusted	0.014 (0.033)	0.58	−0.0026 (0.027)	0.93
Baseline nutritional exposures: estimated intake from FFQ					
Leucine, g/1,000 kcal, from FFQ	Unadjusted	0.053 (0.033)	0.11	0.071 (0.035)	0.04
	Adjusted	0.036 (0.03)	0.23	0.039 (0.036)	0.28
Carbohydrate, g/1,000 kcal, from FFQ	Unadjusted	−0.0005 (0.0009)	0.57	−0.0013 (0.001)	0.17
	Adjusted	−0.0006 (0.0011)	0.59	−0.0008 (0.0013)	0.54

*Adjusted model includes demographics (age, parental education, race, sex, clinic), diabetes-related variables (duration, insulin regimen, insulin dose per kg, fasting glucose, HbA_{1c}), and saturated fat (plasma saturated fatty acids, except for models of exposures based on the FFQ in which saturated fat [grams] from the FFQ was used). †Sample size varies by exposure and model covariates. ‡Step-down tests for breastfeeding history are not provided because the overall tests are not significant.

autoimmunity (35). However, significantly reduced risk of development of islet autoimmunity was associated with increased intake of omega-3 fatty acids among youth genetically susceptible to development of type 1 diabetes (36), although continued follow-up did not reveal subsequent protection against incident type 1 diabetes (37). Our study suggests that long-chain omega-3 fatty acids may provide protection to the β-cell after clinical diagnosis of type 1 diabetes.

Current nutritional exposures: estimated intake from FFQ

Leucine and other branched-chain amino acids have been shown to stimulate insulin secretion through multiple effects on β-cell function (5), as demonstrated in models of type 2 diabetes and in healthy humans. Whether any of these mechanisms are relevant to the capacity of β-cells to continue to produce insulin during the autoimmune process of type 1 diabetes has not been evaluated. Our results support further study of the potential role of branched-chain amino acids to enhance sustained insulin production in type 1 diabetes.

Strengths and limitations

SEARCH and SNAS have considerable strengths, including the availability of detailed nutritional data regarding a large cohort of well-characterized youth with type 1 diabetes and the capacity to evaluate the hypothesized associations in a prospective design. However, because SEARCH is a large epidemiologic study,

Table 3—*Longitudinal associations of baseline nutritional exposures evaluated separately with follow-up log fasting C-peptide for SEARCH 2002–2005 incident cases with preserved β-cell function at baseline, unadjusted and adjusted* for potential confounders*

		Longitudinal analytic subset (baseline FCP >0.23 ng/mL) $N = 656$†	
	Model	Beta coefficient (SE)	*P*
Infant feeding exposures			
Breastfeeding	Unadjusted		0.10 (overall)‡
	Never	−0.05 (0.09)	
	<6 months	0.14 (0.08)	
	≥6 months	0 (referent)	
	Adjusted		0.06 (overall)‡
	Never	−0.08 (0.13)	
	<6 months	0.22 (0.12)	
	≥6 months	0 (referent)	
Age at introduction of complementary food, months			
Any solid food	Unadjusted	0.009 (0.016)	0.56
	Adjusted	0.021 (0.022)	0.35
Any dairy (including formula)	Unadjusted	0.007 (0.0089)	0.42
	Adjusted	0.016 (0.0128)	0.2
Any vegetable (excluding potatoes)	Unadjusted	−0.012 (0.0163)	0.48
	Adjusted	0.003 (0.0218)	0.88
	Adjusted	0.0063 (0.0171)	0.71
Baseline nutritional exposures: biomarkers			
Vitamin D (plasma 25-OHD), nmol/L	Unadjusted	−0.0027 (0.001)	0.005
	Adjusted	−0.004 (0.0013)	0.002
Vitamin E (plasma α-tocopherol), μg/mL	Unadjusted	0.012 (0.0126)	0.33
	Adjusted	0.008 (0.0146)	0.59
EPA, plasma phospholipid percent weight	Unadjusted	0.398 (0.1523)	0.009
	Adjusted	0.353 (0.1453)	0.02
DHA, plasma phospholipid percent weight	Unadjusted	0.127 (0.0498)	0.01
	Adjusted	0.088 (0.0495)	0.08
EPA + DHA	Unadjusted	0.118 (0.041)	0.005
	Adjusted	0.088 (0.041)	0.03
Baseline nutritional exposures: estimated intake from FFQ			
Leucine, g/1,000 kcal, from FFQ	Unadjusted	0.07 (0.0471)	0.14
	Adjusted	0.108 (0.0509)	0.03
Carbohydrate, g/1,000 kcal, from FFQ	Unadjusted	−0.0005 (0.0013)	0.71
	Adjusted	−0.0015 (0.0018)	0.41

Baseline FCP >0.23 ng/mL. *Adjusted model includes demographics (age, parental education, race, sex, clinic), diabetes-related variables (duration, insulin regimen, insulin dose per kg, fasting glucose, HbA_{1c}), and saturated fat (plasma saturated fatty acids, except for models of exposures based on the FFQ in which saturated fat [grams] from the FFQ was used). †Sample size varies by exposure and model covariates. ‡Step-down tests for breastfeeding history are not provided because the overall tests are not significant.

we used fasting, rather than stimulated, C-peptide levels to assess β-cell function. Although FCP correlates well with stimulated C-peptide (38), future studies would benefit from use of outcomes derived from standardized mixed meal–stimulated C-peptide. Exposure assessment was imperfect, partly because of reliance on recall by mothers (for infant feeding) or self-report for baseline dietary intake. However, recall bias is unlikely given that nutrition is not a commonly recognized correlate of FCP among youth with established type 1 diabetes. Further, although analyses adjusted for diabetes duration and length of follow-up, the baseline assessment was not completed immediately on diagnosis, and duration of follow-up was variable. It does not seem likely that bias would result from these time-related design issues; however, some degree of precision may have been lost. Finally, despite the study a priori hypotheses driven by biologic plausibility and previous literature, the observed statistically significant findings may be attributable to chance. Our findings suggest that selected nutritional factors, particularly intake of long-chain omega-3 fatty acids and branched-chain amino acids, may have a positive impact on β-cell function in a clinically relevant manner. Mechanisms may relate to those postulated for development of diabetes-related autoimmunity or incidence of type 1 diabetes, or may be unique to the natural history of β-cell failure in the face of autoimmunity after clinical diagnosis of type 1 diabetes. These novel results can be used to design future studies to establish the efficacy and effectiveness of nutritional approaches to support preservation of β-cell function among youth with recently diagnosed type 1 diabetes.

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E.J.M.-D. wrote the manuscript and researched the data. D.D. contributed to the discussion and reviewed and edited the manuscript. J.L.C. researched the data and reviewed and edited the manuscript. T.C. reviewed and edited the manuscript. R.B.D. reviewed and edited the manuscript. L.D. reviewed and edited the manuscript. I.B.K. reviewed and edited the manuscript. J.M.L. reviewed and edited the manuscript. J.M.N. reviewed and edited the manuscript and contributed to discussion. C.P. reviewed and edited the manuscript. N.T. reviewed and edited the manuscript and contributed to discussion. E.J.M.-D. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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References

1. Greenbaum CJ, Anderson AM, Dolan LM, et al.; SEARCH Study Group. Preservation of beta-cell function in autoantibody-positive youth with diabetes. Diabetes Care 2009;32:1839–1844
2. Palmer JP, Fleming GA, Greenbaum CJ, et al. C-peptide is the appropriate outcome measure for type 1 diabetes clinical trials to preserve beta-cell function: report of an ADA workshop, 21-22 October 2001. Diabetes 2004;53:250–264
3. Steffes MW, Sibley S, Jackson M, Thomas W. Beta-cell function and the development of diabetes-related complications in the Diabetes Control and Complications Trial. Diabetes Care 2003;26:832–836
4. Virtanen SM, Knip M. Nutritional risk predictors of beta cell autoimmunity and type 1 diabetes at a young age. Am J Clin Nutr 2003;78:1053–1067
5. Yang J, Chi Y, Burkhardt BR, Guan Y, Wolf BA. Leucine metabolism in regulation of insulin secretion from pancreatic beta cells. Nutr Rev 2010;68:270–279
6. Virtanen SM, Hyppönen E, Läärä E, et al.; Childhood Diabetes in Finland Study Group. Cow's milk consumption, disease-associated autoantibodies and type 1 diabetes mellitus: a follow-up study in siblings of diabetic children. Diabet Med 1998;15:730–738
7. Norris JM, Scott FW. A meta-analysis of infant diet and insulin-dependent diabetes mellitus: do biases play a role? Epidemiology 1996;7:87–92
8. Norris JM, Barriga K, Klingensmith G, et al. Timing of initial cereal exposure in infancy and risk of islet autoimmunity. JAMA 2003;290:1713–1720
9. Ziegler AG, Schmid S, Huber D, Hummel M, Bonifacio E. Early infant feeding and risk of developing type 1 diabetes-associated autoantibodies. JAMA 2003;290:1721–1728
10. Stene LC, Joner G; Norwegian Childhood Diabetes Study Group. Use of cod liver oil during the first year of life is associated with lower risk of childhood-onset type 1 diabetes: a large, population-based, case-control study. Am J Clin Nutr 2003;78:1128–1134
11. Knekt P, Reunanen A, Marniemi J, Leino A, Aromaa A. Low vitamin E status is a potential risk factor for insulin-dependent diabetes mellitus. J Intern Med 1999;245:99–102
12. Nuttall FQ. Carbohydrate and dietary management of individuals with insulin-requiring diabetes. Diabetes Care 1993;16:1039–1042
13. SEARCH Study Group. SEARCH for Diabetes in Youth: a multicenter study of the prevalence, incidence and classification of diabetes mellitus in youth. Control Clin Trials 2004;25:458–471
14. Bonifacio E, Yu L, Williams AK, et al. Harmonization of glutamic acid decarboxylase and islet antigen-2 autoantibody assays for National Institute of Diabetes and Digestive and Kidney Diseases consortia. J Clin Endocrinol Metab 2010;95:3360–3367
15. Erlich H, Valdes AM, Noble J, et al.; Type 1 Diabetes Genetics Consortium. HLA DR-DQ haplotypes and genotypes and type 1 diabetes risk: analysis of the type 1 diabetes genetics consortium families. Diabetes 2008;57:1084–1092
16. Mayer-Davis EJ, Nichols M, Liese AD, et al.; SEARCH for Diabetes in Youth Study Group. Dietary intake among youth with diabetes: the SEARCH for Diabetes in Youth Study. J Am Diet Assoc 2006;106:689–697
17. Kostraba JN, Cruickshanks KJ, Lawler-Heavner J, et al. Early exposure to cow's milk and solid foods in infancy, genetic predisposition, and risk of IDDM. Diabetes 1993;42:288–295
18. Hollis BW. Editorial: The determination of circulating 25-hydroxyvitamin D: no easy task. J Clin Endocrinol Metab 2004;89:3149–3151
19. Cheng TY, Barnett MJ, Kristal AR, et al. Genetic variation in myeloperoxidase modifies the association of serum α-tocopherol with aggressive prostate cancer among current smokers. J Nutr 2011;141:1731–1737
20. Ma J, Folsom AR, Shahar E, Eckfeldt JH; The Atherosclerosis Risk in Communities (ARIC) Study Investigators. Plasma fatty acid composition as an indicator of habitual dietary fat intake in middle-aged adults. Am J Clin Nutr 1995;62:564–571
21. King IB, Lemaitre RN, Kestin M. Effect of a low-fat diet on fatty acid composition in red cells, plasma phospholipids, and cholesterol esters: investigation of a biomarker of total fat intake. Am J Clin Nutr 2006;83:227–236
22. Tacconi M, Wurtman RJ. Rat brain phosphatidyl-N,N-dimethylethanolamine is rich in polyunsaturated fatty acids. J Neurochem 1985;45:805–809
23. Lemaitre RN, King IB, Patterson RE, Psaty BM, Kestin M, Heckbert SR. Assessment of trans-fatty acid intake with a food frequency questionnaire and validation with adipose tissue levels of trans-fatty acids. Am J Epidemiol 1998;148:1085–1093

24. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr 2000;72:690–693
25. Dabelea D, Mayer-Davis EJ, Andrews JS, et al. Clinical evolution of beta cell function in youth with diabetes: the SEARCH for Diabetes in Youth study. Diabetologia 2012;55:3359–3368
26. Vaarala O, Knip M, Paronen J, et al. Cow's milk formula feeding induces primary immunization to insulin in infants at genetic risk for type 1 diabetes. Diabetes 1999;48:1389–1394
27. Hummel S, Pflüger M, Hummel M, Bonifacio E, Ziegler AG. Primary dietary intervention study to reduce the risk of islet autoimmunity in children at increased risk for type 1 diabetes: the BABYDIET study. Diabetes Care 2011;34:1301–1305
28. Borges MC, Martini LA, Rogero MM. Current perspectives on vitamin D, immune system, and chronic diseases. Nutrition 2011;27:399–404
29. Hyppönen E, Läärä E, Reunanen A, Järvelin MR, Virtanen SM. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. Lancet 2001;358:1500–1503
30. Simpson M, Brady H, Yin X, et al. No association of vitamin D intake or 25-hydroxyvitamin D levels in childhood with risk of islet autoimmunity and type 1 diabetes: the Diabetes Autoimmunity Study in the Young (DAISY). Diabetologia 2011;54:2779–2788
31. Bizzarri C, Pitocco D, Napoli N, et al; IMDIAB Group. No protective effect of calcitriol on beta-cell function in recent-onset type 1 diabetes: the IMDIAB XIII trial. Diabetes Care 2010;33:1962–1963
32. Walter M, Kaupper T, Adler K, Foersch J, Bonifacio E, Ziegler AG. No effect of the 1alpha,25-dihydroxyvitamin D3 on beta-cell residual function and insulin requirement in adults with new-onset type 1 diabetes. Diabetes Care 2010;33:1443–1448
33. Sijben JW, Calder PC. Differential immunomodulation with long-chain n-3 PUFA in health and chronic disease. Proc Nutr Soc 2007;66:237–259
34. Krishna Mohan I, Das UN. Prevention of chemically induced diabetes mellitus in experimental animals by polyunsaturated fatty acids. Nutrition 2001;17:126–151
35. Virtanen SM, Niinistö S, Nevalainen J, et al. Serum fatty acids and risk of advanced beta-cell autoimmunity: a nested case-control study among children with HLA-conferred susceptibility to type I diabetes. Eur J Clin Nutr 2010;64:792–799
36. Norris JM, Yin X, Lamb MM, et al. Omega-3 polyunsaturated fatty acid intake and islet autoimmunity in children at increased risk for type 1 diabetes. JAMA 2007;298:1420–1428
37. Miller MR, Yin X, Seifert J, et al. Erythrocyte membrane omega-3 fatty acid levels and omega-3 fatty acid intake are not associated with conversion to type 1 diabetes in children with islet autoimmunity: the Diabetes Autoimmunity Study in the Young (DAISY). Pediatr Diabetes 2011;12:669–675
38. Raz I, Elias D, Avron A, Tamir M, Metzger M, Cohen IR. Beta-cell function in new-onset type 1 diabetes and immunomodulation with a heat-shock protein peptide (DiaPep277): a randomised, double-blind, phase II trial. Lancet 2001;358:1749–1753